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Research Article

ANALYSIS OF HIGH DOSE AND LONG-TERM PREDNISONE THERAPY ON OSTEOCALCIN LEVELS IN CHILDREN WITH NEPHROTIC SYNDROME (STUDY AT PEDIATRIC DEPARTMENT NEPHROLOGY DIVISION OF DR. SOETOMO TEACHING HOSPITAL SURABAYA)

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ABSTRACT

Objective: The objective of the study to analyze osteocalcin levels in induction and alternate phase, associated with clinical manifestation.

Methods: We conducted a prospective longitudinal study. This study was subject to approval by the Ethics Committee of Dr. Soetomo Teaching Hospital Surabaya. Osteocalcin levels were measured before and after induction phases and 4 weeks after the alternate phase. Samples were collected in the morning at 08.00-09.00 am.

Results: A total of 15 patients were included in this study. The suppression of osteocalcin levels in the induction phase was 53.33%. After the alternate phase, osteocalcin levels increased 175.82%. Clinical manifestation as bone pain/cramps only appeared 33% in the induction phase and 20% in the alternate phase. The mean suppression of osteocalcin levels in the group with induction phase duration therapy ≥28 days and without calcium supplementation was higher than 21-27 days and with calcium supplementation. Osteocalcin levels increased in the alternate phase also in patients with and without calcium supplementation. The result of analysis showed there was no significant difference among all groups ($p>0.05$).

Conclusion: Suppression of osteocalcin levels was reversible after the alternate phase. It shows that tapering off regimen is important. Clinical sign as pain bone/cramps almost showed no manifest in all of these patients.

Keywords: Osteocalcin, Nephrotic syndrome, Prednisone, Corticosteroid, High dose prednisone, Long-term prednisone, Bone formation, Bone marker, Pediatric.

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INTRODUCTION

Nephrotic syndrome (NS) is a glomerular disease with massive proteinuria >40 mg/m^2/h or >50 mg/kg/d or urinary protein creatinine ratio >2 mg/mg. Other clinical manifestations are hypoalbuminemia (≤2.5 g/dl) and edema [1-3]. Based on the report, NS incident occurrence of children <14 year of age in Indonesia is 6/100.000 [2]. NS patients are treated with high dose and long-term prednisone therapy which may decrease the function of osteoblasts and lead to suppression of bone formation [4,5]. A meta-analysis of over 80 studies in adults found that use of ≥5 mg/d of prednisolone (or equivalent) was associated with significant reductions in bone mineral density (BMD) and an increase in fracture risk within 3-6 months of treatment initiation [6]. Based on systematic review and meta-analysis in children ≤18 years taking long-term systemic glucocorticoid therapy, the prevalence of fracture was 29-45% [7]. Based on those meta-analysis, NS patients are at risk of losing bone mass which leads to osteopenia, osteoporosis, and fracture. Therefore, bone metabolism in NS patients need to be evaluated.

Biochemical marker such as osteocalcin may be useful in monitoring bone metabolism. Osteocalcin is a noncollagenous protein in mature human bone. It is a small protein of 49 amino acids including three residues of gamma-carboxyglutamic acid and its synthesis is essentially specific to osteoblasts [8,9]. Osteocalcin is a sensitive marker to glucocorticoids because it directly affects the expression of osteocalcin at the transcriptional level by binding to the glucocorticoid receptors (GR) and leading to repression of osteocalcin gene up to 40% of basal levels [10,11]. Osteocalcin levels of children with NS in Indonesia have not been established. Besides that, osteocalcin levels after 4 weeks the alternate phase in NS patients have not been evaluated. Therefore, this study is proposed to analyze osteocalcin levels in the induction phase and 4 weeks of the alternate phase which associated with clinical manifestation.

METHODS

We conducted a prospective longitudinal study from May to October 2016. This study protocol was subject to approval by the Ethics Committee of Dr. Soetomo Teaching Hospital Surabaya. We included children aged <18 years who were diagnosed with an initial attack NS, infrequent relapses NS, frequently relapsing NS, and steroid dependent NS. The parents of the patients signed informed consents for this study. The patients with NS steroid resistant were excluded from this study.

NS patients were treated with 60 mg/m^2/d prednisone or 2 mg/kg/d (maximum 60 mg/d) for ±4 weeks (induction phase), followed by 40 mg/m^2/d or 1.5 mg/kg/d (maximum 40 mg/d) on alternate days for ±4 weeks (alternate phase). Samples were collected at 08.00-09.00 am from the patients before they started treatment, after the induction phase, and after alternate phase. All patients had normal renal function (glomerular filtration rate >90 ml/minutes/1.73 m^2).

Osteocalcin levels were measured accordance with Enzyme-Linked Immunosorbent Assay method. Medical history and clinical data were collected from patient information and based on the medical record.

Nominal data were presented as frequency distribution, and interval data were described as mean and standard deviation. Collected data were analyzed using Statistical Package for the Social Science 20,0. Distribution data were checked with One-Sample Kolmogorov-Smirnov

Table 1: Baseline characteristics of patients

Patient characteristics	Total patient (n=15)	Mean±SD
	Number of patients n (%)	
Gender		
Boys	9 (60)	-
Girls	6 (40)	-
Age (year)		
<2	0 (0)	-
2-<	4 (27)	3.5±1.3
6-<	8 (53)	7.5±1.6
12-18	3 (20)	14±1.0
Weight (kg)		
≤20.9	8 (53)	16.4±3.13
21-40.9	5 (33)	26.9±8.05
41-60	2 (14)	48.5±2.12
Diagnosis		
Initial attack nephrotic syndrome	4 (27)	-
Infrequent relapses nephrotic syndrome	3 (20)	-
Frequently relapsing nephrotic syndrome	2 (13)	-
Steroid dependent nephrotic syndrome	6 (40)	-
Osteocalcin level before induction (µg/l)		
<45	4 (27)	19.151±15.358
45-105	4 (27)	70.923±22.976
>105	7 (46)	134.623±11.526
Cumulative dose of prednisone 1 year before induction (g)		
Not taking prednisone	5 (33)	-
<5	6 (40)	3.1±1.39
5-10	4 (37)	5.9±5.2
>10	0 (0)	-
Duration of prednisone 1 year before induction (days)		
Not taking prednisone	5 (33)	-
1-225	9 (60)	168.2±53.96
226-450	1 (7)	229
>450	0 (0)	-
Cumulative dose of calcium supplement 1 year before induction (g)		
Not taking calcium supplement	6 (40)	-
<293	2 (13)	175.3±148.14
293-586	7 (47)	327.6±17.29
Calcium level before induction		
No measurement	2 (13)	-
Normal (2.2-2.7 mmol/l)	13 (87)	2.3±0.13
Abnormal (<2.2 or>2.7 mmol/l)	0 (0)	-
Phosporus level before induction		
No measurement	7 (47)	-
Normal (0.87-2.1 mmol/l)	8 (53)	1.6±0.21
Abnormal (<0.87 or>2.1 mmol/l)	0 (0)	-
Edema		
No edema	1 (7)	-
Palpebra	4 (26)	-
Extremitas	1 (7)	-
Anasarca	9 (60)	-
Bone pain/cramps		
Yes	5 (33)	-
No	10 (67)	-

SD: Standard deviation

test. Differences in osteocalcin levels between phase were checked with Paired t-test and General Linear Model Repeated Measures. Independent t-test and One-Way ANOVA were used to compare differences in osteocalcin levels among groups. For all test, a probability, $p<0.05$ was examined significant.

RESULTS

A total of 15 patients (9 boys and 6 girls) were enrolled from Pediatric Department Nephrology Division of Dr. Soetomo Teaching Hospital Surabaya. The patients were in the active phase of NS, with either their first attack (n=4) or during a relapse (n=11). Their ages ranged from 2 to 15 years with most of the weight were ≤20.9 kg. Before induction phase, 27% of patient had osteocalcin level in the normal range, 47% up in the normal range, and 27% below the normal range. The majority of the patients had normal of calcium and phosphorus levels, also did not experience bone pains/cramps. Table 1 shows baseline characteristics of examined children and history of the duration and cumulative dose of prednisone for 1 year before induction (t=0).

Osteocalcin levels of patients before treatment started (t=0), after induction phase (t=1), and after alternate phase (t=2) were shown in Table 2. Almost all patients had osteocalcin levels at t=0 higher than the normal range, except four patients (AM, KY, AP, and RA) whose osteocalcin levels were lower than normal range. Other than KY, osteocalcin levels at t=2 were higher than the normal range. Three patients (MA infrequent relapses NS, AP frequently relapsing NS, RA-dependent steroid NS) had osteocalcin profile different from other patients, which osteocalcin levels at t=0 lower than t=1. Osteocalcin suppression of DA in the induction phase was as low as 2.44% compared

Table 2: Osteocalcin levels of patients

Patients code	Osteocalcin levels (µg/l)			
	Normal range	**t=0**	**t=1**	**t=2**
Initial attack nephrotic syndrome				
AM (G/3 y/11.8 kg)	44-93	37.892	16.931	63.635
DI (G/8 y/20 kg)	50-89	137.321	34.434	141.462
KY (B/2 y/12.3 kg)	64-70	24.274	2.458	34.818
AI (B/14 y/47 kg)	66-102	146.713	30.976	180.370
Mean±SD		86.550±64.403[1*]	21.200±14.608[2*]	105.071±67.445[3*]
(%)		-	↓75.51%	↑395.62%
Infrequent relapses nephrotic syndrome				
JA (G/7 y/18 kg)	53-97	138.218	39.088	124.855
SR (G/7 y/21.8 kg)	53-97	128.356	10.740	63.806
MA (B/7 y/20.2 kg)	49-104	49.411	118.174	145.386
Mean±SD		105.328±48.676[1*]	56.001±55.678[2*]	111.349±42.434[3*]
%		-	↓46.83%	↑98.83%
Frequently relapsing nephrotic syndrome				
AR (B/6 y/16.1 kg)	51-84	114.762	51.700	141.023
AP (B/11 y/50 kg)	56-80	2.458	7.667	46.174
Mean±SD		58.610±79.411[1*]	29.684±31.136[2*]	93.599±67.068[3*]
%		-	↓49.35%	↑215.32%
Steroid dependent nephrotic syndrome				
RF (B/15 y/29.7 kg)	61-105	103.339	32.342	132.283
ND (B/4 y/16 kg)	53-90	63.166	40.753	128.825
RA (B/6 y/17 kg)	51-84	11.978	21.029	56.164
DA (G/5 y/21 kg)	66-88	129.361	126.208	142.190
NH (G/8 y/22 kg)	50-89	67.776	45.235	136.766
FH (B/13 y/39.8 kg)	74-98	147.632	30.233	139.123
Mean±SD		87.209±49.605[1*]	49.300±38.613[2*]	122.559±32.875[3*]
%		-	↓43.47%	↑148.60%
Mean±SD (15 patients)		86.844±52.380[4*]	40.531±36.030[4*5*]	111.792±45.268[5*]
%		-	↓53.33	↑175.82
Range		2.458-147.632	2.458-126.208	34.818-180.370

[1*]p=0.847, [2*]p=0.572, [3*]p=0.889, [4*]p=0.007 (t=0 vs. t=1), [5*]p=0.000 (t=1 vs. t=2), G: Girl, B: Boy, SD: Standard deviation, y: Years

to high percentage of other patients. There was no significant difference in osteocalcin level of initial attack, infrequent relapses, frequently relapsing, and dependent steroid NS at t=0, t=1, t=2 (p=0.847; p=0.572; p=0.889, respectively).

The mean osteocalcin level of 15 patients was presented in Table 2 showed that after the induction phase, osteocalcin levels decrease 53.33% (86.844±52.380 to 40.531±36.030 µg/l) and after alternate phase increase 175.82% (40.531±36.030 to 111.792±45.268 µg/l). There was a significant difference between osteocalcin levels after induction phase versus (vs.) before treatment and after alternate phase versus induction phase (p=0.007 and p=0.000, respectively).

Data in Table 3 that showed the mean suppression of osteocalcin levels in group with induction phase duration therapy ≥28 days and without calcium supplementation was higher than 21-27 days and with calcium supplementation (−56.752±58.674 and −64.467±67.921 vs. −25.434±51.932 and −30.376±43.166) µg/l, respectively. Osteocalcin levels increased in the alternate phase also in patients with and without calcium supplementation. The result of analysis showed that there was no significant difference among all groups (p>0.05).

Clinical manifestation as bone pain/cramps only appeared 33% in the induction phase and 20% in the alternate phase showed in Table 4.

DISCUSSION

Osteocalcin levels of 7 out of 15 patients at t=0 was higher than the normal range. As shown in Table 2 indicate that children in growth period associated with increased activity of osteoblast [8]. Normal range is osteocalcin levels in Caucasian children. The difference of these ethnic may be caused difference of BMD. Based on the report, the prevalence of vitamin D deficiency in people who live in high-latitude regions, such as Caucasian children was higher than people in tropical regions like Indonesian children [12]. Deficiency of vitamin D will be

Table 3: Osteocalcin change based on duration therapy and consume of calcium supplementation

Group	Number of patients	Mean±SD of osteocalcin change (µg/l)	Significancy (p)
Induction phase 21-27 days	5	−25.434±51.932	0.332
Induction phase≥28 days	10	−56.752±58.674	
Alternate phase≤14 days	6	83.682±23.654	0.325
Alternate phase>14 days	9	62.980±45.191	
With calcium supplement (induction phase)	8	−30.376±43.166	0.261
Without calcium supplement (induction phase)	7	−64.467±67.921	
With calcium supplement (alternate phase)	10	58.547±30.793	0.067
Without calcium supplement (alternate phase)	5	96.688±42.694	

SD: Standard deviation

Table 4: Clinical manifestation

Patient code	Bone pain/cramp		
	t=0	t=1	t=2
Initial attack nephrotic syndrome			
AM	No	No	No
DI	No	No	No
KY	No	No	No
AI	No	Yes	No
Infrequent relapses nephrotic syndrome			
JA	Yes	No	Yes
SR	No	No	No
MA	Yes	Yes	No
Frequently relapsing nephrotic syndrome			
AR	No	Yes	No
AP	Yes	No	No
Steroid dependen nephrotic syndrome			
RF	No	No	No
ND	No	Yes	No
RA	Yes	No	Yes
DA	Yes	No	No
NH	No	No	No
FH	No	Yes	Yes
Total (%)	Yes=5 (33) No=10 (67)	Yes=5 (33) No=10 (67)	Yes=3 (20) No=12 (80)

reduced transcription of osteocalcin; therefore, osteocalcin level in most patients was higher than normal range [13]. Difference of ethnic also might cause genetic variation, polymorphisms in the promoter gene of osteocalcin (rs1800247), vitamin D receptor gene, and regulatory region of the type 1 collagen gene, COLIA1, also mutation of low-density lipoprotein receptor-related protein 5 (LRP5) therefore may cause difference osteocalcin levels [14-16]. Four patients (AM, KY, AP, and RA) whose osteocalcin levels lower than normal range may be caused by low quality of BMD in these patients.

Three patients (MA infrequent relapses NS, AP frequently relapsing NS, RA-dependent steroid NS) had osteocalcin profile different from other patients, whose osteocalcin levels at t=0 was lower than t=1 which caused by a history of high-dose and long-term prednisone since 2015, 2013, and 2011, respectively. The suppression osteocalcin of DA in the induction phase as low as 2.44% because this patient had high protein intake. Protein intake affects bone in several ways, such as providing the structural matrix of bone, optimizing insulin-like growth factor-1 levels, and reported increasing intestinal calcium absorption [17].

Osteocalcin levels of initial attack, infrequent relapses, frequently relapsing, and dependent steroid NS at t=0, t=1, t=2 were not significantly different because patients were given the same dose. Kano *et al.* [18] reported osteocalcin levels of children with steroid-responsive NS 4 weeks after starting prednisolone therapy (induction phase) decreased 68.54%. The other study that was reported by Mohamed and Abdul-Latif [19], osteocalcin levels decreased 30.59% after 1 month of therapy in the initial attack NS. The decreasing of osteocalcin levels after induction phase also occurred in 15 patients in this study. Osteocalcin levels of 15 patients decrease 53.33% (86.844±52.380 - 40.531±36.030 µg/l). It showed a decreasing function of osteoblast which leads to osteopenia, osteoporosis, and increasing risk of fracture. This suppression was caused by prednisone induce nuclear factors of the CCAAT enhancer binding protein family and the induction of peroxisome proliferator-activated receptor γ 2, both of which play essential roles in adipogenesis. An additional mechanism by which prednisone inhibit osteoblast cell differentiation is by opposing Wnt/β-catenin signaling. Prednisone has pro-apoptotic effects on osteoblasts and osteocytes due to activation of caspase 3, a common downstream effector of several apoptotic signaling pathways [4,5]. Besides that, prednisone has directly affected the expression of osteocalcin at the transcriptional level by binding to the GR and subsequently to specific recognition sequences in the proximal promoter leading to a repression of osteocalcin gene [10].

Kano *et al.* [18] observed osteocalcin levels 8 weeks after the alternate phase and showed increasing from 6.7±2.6 µg/l to 15.0±6.4 µg/l. As mentioned previously that study of osteocalcin levels after 4 weeks the alternate phase in NS patients have not been evaluated. Therefore, our study evaluated it and the result showed increasing 175.82% (40.531±36.030 - 111.792±45.268 µg/l). This increasing indicates that the suppression of osteocalcin is reversible while tapering dose of prednisone.

The mean suppression of osteocalcin levels in the group with induction phase duration therapy ≥28 days was higher than 21-27 days. This condition as reported Godschalk and Downs [20] and Biyikli *et al*. [21] that the suppression of osteocalcin is dose dependent, which the higher dose of prednisone, the higher suppression of osteocalcin levels. Patients without calcium supplement had osteocalcin levels more suppressed than with calcium supplement. This result shows that patients without calcium supplement are at risk of hypocalcemia, thus stimulates parathyroid hormone (PTH). PTH acts on osteoclast by increasing receptor activator of NF-κB ligand release, leading to promotion of osteoclastogenesis and reduce osteoblastogenesis, therefore suppress transcription of osteocalcin [22]. It shows that calcium supplement should be given to prevent hypocalcemia in NS patient.

Variation on increasing of osteocalcin levels in the alternate phase is affected by BMD each patient and intake patients. Dietary such as milk, fish, egg, and green leaves (spinach, broccoli) is intake high calcium, protein, vitamin D, and vitamin K which may increase transcription of osteocalcin [23,24].

CONCLUSION

Suppression of osteocalcin levels was reversible after alternate phase. It shows that tapering off regimen is important. Clinical sign as pain bone/cramps almost showed no manifest in all of these patients.

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